

Inventors: Doekel et al. Serial No. 09/966,742

**REMARKS**

Upon entry of this Amendment, claims 1-25 will be pending, of which claims 1 and 2 are independent. The claims have been amended to eliminate multiple dependencies and to employ more conventional U.S. claim language. In addition, the specification has been amended to include a cross reference to the PCT parent application. It is respectfully submitted that no new matter has been introduced.

It is respectfully submitted that the Application is in condition for allowance and a Notice to that effect is courteously solicited. If any questions remain, however, the Examiner is encouraged to call undersigned to expedite the prosecution of this Application.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By: Richard A. Steinberg  
Richard A. Steinberg  
Registration No. 26,588  
Paul L. Sharer  
Registration No. 36,004

1600 Tysons Boulevard  
McLean, VA 22102  
(703) 905-2000 Telephone  
(703) 905-2500 Facsimile

**APPENDIX**

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE SPECIFICATION:**

A cross-reference to the PCT parent application has been added.

**IN THE CLAIMS:**

**The claims are amended as follows:**

1. (Amended) Method for the microbiological production of  $\alpha$ -L-aspartyl-L-phenylalanine (Asp-Phe) from the substrates L-aspartic acid (L-Asp) and L-phenylalanine (L-Phe) [characterised in that] wherein the substrates are contacted, in the presence of an effective amount of adenosine-triphosphate (ATP), with a non-ribosomal dipeptide synthetase comprising two minimal modules connected by one condensation domain<sub>2</sub> wherein the N-terminal module of these modules is recognising L-aspartic acid and the C-terminal module of these modules is recognising L-phenylalanine and is covalently bound at its N-terminal end to the condensation domain, and wherein each of these minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain, and that the  $\alpha$ -L-aspartyl-L-phenylalanine (Asp-Phe) formed is recovered.
2. (Amended) Method for the production of Asp-Phe according to claim 1, [characterised in that] wherein the condensation domain in the dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.

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3. (Amended) Method for the production of Asp-Phe according to claim 1<sub>2</sub> [or 2, characterised in that also] further comprising a thioesterase-like releasing factor [is present] for the Asp-Phe formed on the dipeptide synthetase.
4. (Amended) Method for the production of Asp-Phe according to [any of claims 1 to 3, characterised in that] claim 1, wherein the thioesterase-like releasing factor forms an integrated domain of the dipeptide synthetase at the C-terminus thereof.
5. (Amended) Method for the production of Asp-Phe according to [any of claims 1 to 4, characterised in that also] claim 1, wherein a non-integrated protein with thioesterase Type-II-like activity is further present together with the dipeptide synthetase.
6. (Amended) Method for the production of Asp-Phe according to [any of claims 1 to 5, characterised in that] claim 5, wherein the dipeptide synthetase is present in living cell-material of a micro-organism[.]; [and that] glucose, L-Asp [and/or], L-Phe, or mixtures thereof are being fed to said fermentor[.]; and [that] the Asp-Phe formed is recovered.
7. (Amended) Method for the production of Asp-Phe according to claim 6<sub>2</sub> [characterised in that] wherein the micro-organism is first grown in a fermentor to reach a predetermined cell density before the expression of the Asp-Phe dipeptide synthetase is switched on<sub>2</sub> and feeding of the glucose, L-Asp<sub>2</sub> [and/or] L-Phe, or mixtures thereof for the synthesis of the Asp-Phe dipeptide is started.
8. (Amended) Method for the production of Asp-Phe according to claim 7, [characterised in that] wherein the micro-organism is an L-phenylalanine producing micro-organism<sub>2</sub> and [that] only glucose and L-Asp are being fed.
9. (Amended) Method for the production of Asp-Phe according to claim 8, [characterised in that] wherein the micro-organism is an *Escherichia* or *Bacillus* species.

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10. (Amended) Method for the production of Asp-Phe according to [any of claims 6 to 9, characterised in that] claim 6, wherein the micro-organism used is a strain [with] having reduced protease activity for Asp-Phe or [lacking such] having no protease activity towards Asp-Phe.
11. (Amended) Method for the production of Asp-Phe according to [any of claims 1 to 5, characterised in that] claim 1, wherein the production of Asp-Phe is carried out *in vitro* in an enzyme reactor, while ATP is supplied, [and] L-Asp [and/or] L-Phe, or mixtures thereof is [are] being fed, and the Asp-Phe formed is recovered.
12. (Amended) Method for the production of Asp-Phe according to claim 11, [characterised in that] wherein the supply of ATP is provided in part by an in situ ATP-regenerating system.
13. (Amended) Method for the production of Asp-Phe according to claim 12, [characterised in that] wherein the ATP-regenerating system is present in a permeabilised micro-organism.
14. (Amended) A DNA fragment or a combination of DNA fragments coding for a non-ribosomal Asp-Phe dipeptide synthetase, [which]said synthetase comprises two minimal modules connected by one condensation domain, wherein the N-terminal module of these modules is recognising L-aspartic acid, and the C-terminal module of these modules is recognising L-phenylalanine; and is covalently bound at its N-terminal end to the condensation domain, and wherein each of [these] said minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain.
15. (Amended) A DNA fragment coding for an Asp-Phe dipeptide synthetase according to claim 14, [characterised in that] wherein the condensation domain in the encoded

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dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.

16. (Amended) A DNA fragment [according to claim 14 or 15,] or a combination of DNA fragments according to claim 14, [characterised in that] wherein the DNA fragment or the combination of DNA fragments encoding the dipeptide synthetase also code for a releasing factor for the Asp-Phe formed on that dipeptide synthetase.
17. (Amended) A DNA fragment or a combination of DNA fragments according to claim 16, [characterized in that] wherein the thioesterase-like releasing factor-forms an integrated domain of the dipeptide synthetase at the C-terminus thereof.
18. (Amended) A DNA fragment or a combination of DNA fragments according to [any of claims 14 to 17, characterised in that it/they] claim 14, wherein said DNA fragment or a combination of DNA fragments also code for a non-integrated protein with thioesterase Type-II-like activity.
19. (Amended) A recombinant micro-organism containing a DNA fragment or a combination of DNA fragments according to [any of claims 14-18] claim 14.
20. (Amended) A micro-organism according to claim 19, wherein the micro-organism is capable of producing L-asp<sub>2</sub> [and/or} L-phe, or mixtures thereof.
21. (Amended) A micro-organism according to claim 25, wherein the micro-organism is an *Escherichia coli* or *Bacillus* species.
22. (Amended) Asp-Phe dipeptide synthetase [characterised in that it comprises] comprising two minimal modules connected by one condensation domain, wherein the N-terminal module of these modules is recognising L-aspartic acid and the C-terminal module of these modules is recognising L-phenylalanine and is covalently bound at its N-terminal

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end to the condensation domain, and wherein each of these minimal modules is composed of an adenylation domain and a 4'-phosphopantetheinyl cofactor containing thiolation domain.

23. (Amended) Asp-Phe dipeptide synthetase according to claim 22, [characterised in that] wherein the condensation domain in the dipeptide synthetase is connected to both minimal modules in such way that it is also covalently bound to the module recognising L-aspartic acid.
24. (Amended) Asp-Phe dipeptide synthetase according to claim 22, [or 23, characterised in that] wherein the dipeptide synthetase also comprises a releasing factor for the Asp-Phe formed on that dipeptide synthetase.
25. (Amended) Asp-Phe dipeptide synthetase according to claim 24, [characterised in that] wherein the releasing factor is a protein which shows thioesterase-like functions and forms an integrated domain of the dipeptide synthetase at its C-terminus.